

Listing of Claims

Claims 1-20 (cancel).

21. (Withdrawn) A method of preparing a recombinant adenovirus (RAdEs) vaccine (ECACC Accession Number 04121701) to protect against Japanese encephalitis virus (JEV) infection, wherein said vaccine produces secretory envelop protein (Es) of JEV, said method comprising the steps of:

- a) digesting plasmid pMEs from Japanese encephalitis virus with restriction enzymes *Kpn I* and *Bam HI* to obtain cDNA encoding JEV proteins prM and Es,
- b) ligating the cDNA to adenovirus shuttle plasmid pShuttle digested with restriction enzymes *Kpn I* and *Hind III* at the *Kpn I* end,
- c) adding nucleotides at the free *Bam HI* and *Hind III* ends with T 4 DNA polymerase to create blunt ends,
- d) ligating the blunt ends together to yield shuttle plasmid pSEs with JEV cDNA encoding the proteins prM and Es,
- e) digesting the shuttle plasmid pSEs with restriction enzymes *I-Ceu I* and *PI-Sce I* to obtain expression cassette containing the JEV cDNA together with the CMV promoter/enhancer and BGH polyadenylation signal,
- f) ligating the digested shuttle plasmid with *I-Ceu I* and *PI-Sce I* digested adenovirus plasmid pAdeno-X to generate plasmid pAdEs containing Es expression cassette,
- g) digesting the plasmid pAdEs with *Pac I*,
- h) transfecting the monolayers HEK 293 cells with digested plasmid pAdEs for about one week, and
- i) obtaining the recombinant virus RAdEs vaccine.

22. (Withdrawn) A method as claimed in claim 21, wherein the transfection is at about 37°C.

23.(Withdrawn) A method as claimed in claim 21, wherein the JEV proteins are under the control of human CMV IE promoter/enhancer.

24. (Currently amended) A recombinant adenovirus (RAdEs) vaccine (ECACC Accession Number 0412101) prepared by the method of claim 21 said a method comprising the steps of :

- a) digesting plasmid pMEs from Japanese encephalitis virus with restriction enzymes *Kpn I* and *Bam HI* to obtain cDNA encoding JEV proteins prM and Es,
- b) ligating the cDNA to adenovirus shuttle plasmid pShuttle digested with restriction enzymes *Kpn I* and *Hind III* at the *Kpn I* end,
- c) adding nucleotides at the free *Bam HI* and *Hind III* ends with T 4 DNA polymerase to create blunt ends,
- d) ligating the blunt ends together to yield shuttle plasmid pSEs with JEV cDNA encoding the proteins prM and Es,
- e) digesting the shuttle plasmid pSEs with restriction enzymes *I-Ceu I* and *PI-Sce I* to obtain expression cassette containing the JEV cDNA together with the CMV promoter/enhancer and BGH polyadenylation signal,
- f) ligating the digested shuttle plasmid with *I-Ceu I* and *PI-Sce I* digested adenovirus plasmid pAdeno-X to generate plasmid pAdEs containing Es expression cassette,
- g) digesting the plasmid pAdEs with *Pac I*,
- h) transfecting the monolayers HEK 293 cells with digested plasmid pAdEs for about one week, and
- i) obtaining the recombinant virus RAdEs vaccine.

25. (Previously presented) A recombinant adenovirus (RAdEs) vaccine (ECACC Accession Number 04121701), comprising JEV Es protein optionally with pharmaceutically acceptable additives.

26. (Previously presented) A vaccine as claimed in claim 24, wherein the vaccine produces secretory envelope protein of JEV.
27. (Previously presented) A vaccine as claimed in claim 25, wherein the vaccine produces secretory envelope protein of JEV.
28. (Canceled)
29. (Canceled)
30. (Previously presented) A vaccine as claimed in claim 24, wherein the vaccine is effective by intramuscular route of administration.
31. (Previously presented) A vaccine as claimed in claim 25, wherein the vaccine is effective by intramuscular route of administration.
32. (Previously presented) A vaccine as claimed in claim 24, wherein the additives are selected from a group comprising alum, gelatin and thiomersal.
33. (Previously presented) A plasmid pAdEs of SEQ ID NO:1.
34. (Withdrawn-Currently amended ) A method of immunizing protecting a subject against Japanese encephalitis virus infection comprising administering a vaccine according to claim 24 to the subject in need thereof.
- 35.(Withdrawn-Currently amended) A method of immunizing protecting a subject against Japanese encephalitis virus infection comprising administering a vaccine according to claim 25 to the subject in need thereof.
36. (Withdrawn) The method according to claim 34 to protect the subject from encephalitis.

37. (Withdrawn) The method according to claim 35 to protect the subject from encephalitis.
38. (Withdrawn) The method according to claim 34 wherein the subject is an animal or human.
39. (Withdrawn) The method according to claim 35 wherein the subject is an animal or human.
40. (Withdrawn) The method according to claim 34 wherein the vaccine activates both humoral and cell-mediated immune response.
41. (Withdrawn) The method according to claim 35 wherein the vaccine activates both humoral and cell-mediated immune response.
42. (Withdrawn) The method according to claim 40 wherein the humoral response to the vaccine comprises IgG1 type of antibody.
43. (Withdrawn) The method according to claim 41 wherein the humoral response to the vaccine comprises IgG1 type of antibody.
44. (Withdrawn) The method according to claim 34 wherein the vaccine leads to high amount of IFN - gamma secretion.
45. (Withdrawn) The method according to claim 35 wherein the vaccine leads to high amount of IFN - gamma secretion.
46. (Withdrawn) The method according to claim 34 wherein the vaccine leads to moderate levels of IL -5 synthesis.
47. (Withdrawn) The method according to claim 35 wherein the vaccine leads to moderate levels of IL -5 synthesis.

48. (Withdrawn) The method according to claim 34 wherein increased amount of the vaccine leads to higher immune response.

49. (Withdrawn) The method according to claim 35 wherein increased amount of the vaccine leads to higher immune response.